NREL-Amoco CRADA Phase 3

Bench Scale Report 1.5

Two-Stage Continuous Cofermentation of Pure Sugars by L1400(pLNH33)

Project Title: Amoco-NREL CRADA with corn fiber

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Objective

(1) Examine the ability of L1400(pLNH33) to ferment glucose and xylose in a two-stage system, where a majority of the glucose will be consumed in the first stage leaving only xylose for use in the second stage; and (2) Determine the product distribution and ethanol yield for each stage and the overall process. (Note: In the rest of the report, L1400(pLNH33) will be referred to as LNH33.)

Background

Because a one stage system cannot mimic the multi-stage configuration of the PDU, it is of interest to test this organism (and its descendants) in a multi-stage system in the laboratory. This would allow the glucose to be consumed in the first stage, hence "forcing" the organism to utilize the xylose as its carbon source in the second stage.

Materials and Methods

Inoculum Preparation

A frozen (-70°C) stock vial of LNH33 was grown in 1% w/v corn steep liquor (CSL), 1% w/v yeast extract, 2% w/v peptone, and 2% w/v xylose at pH 5. The flask contained a total volume of 50 mL in a 250-mL baffled Erlenmeyer flask and was incubated at 30°C with an agitation of 150 rpm. After 24 hours of growth, 10% v/v was transferred to 2% w/v CSL, 1% w/v yeast extract, and 2% w/v xylose at pH 5 for inoculum growth. This flask contained a working volume of 100 mL in a 500-mL baffled Erlenmeyer flask and again was incubated at 30°C with an agitation of 150 rpm. After 18.5 hours of incubation, the culture was used to inoculate the first stage fermentor.

Fermentation Conditions and Configuration

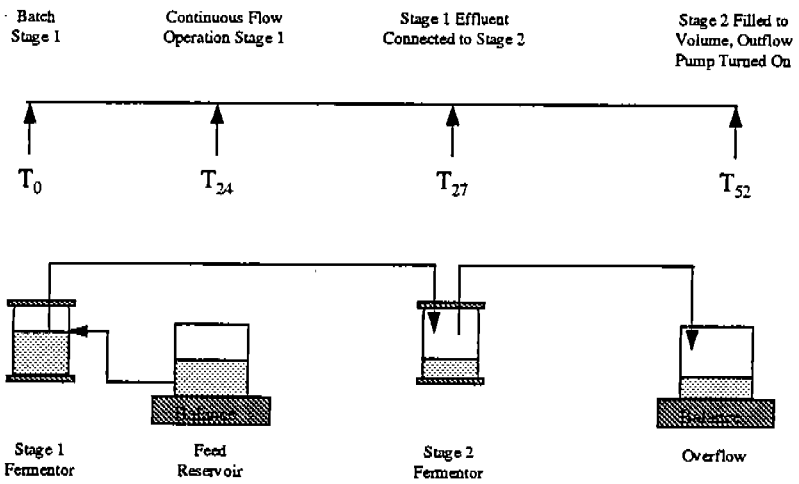
For the fermentations, two 1.7-L New Brunswick BioFlo III fermentors were employed. To minimize ethanol evaporation, the condensers on each unit were packed with 1-mm glass beads (to maximize the surface area) and equipped with 4°C-water circulation. The working volume of each vessel was one liter, agitation was controlled at 150 rpm, temperature was maintained at 30°C, and the pH was maintained at 5.0 with the addition of 3 M sodium hydroxide. Air was not supplied to the fermentors.

The first stage fermentor was started in batch mode with 2% w/v CSL, 1% w/v yeast extract, 2.4% w/v glucose, and 3.4% w/v xylose as the medium. The first stage fermentor was prepared and autoclaved with CSL, water, and yeast extract at pH 5.0. Stock solutions of glucose and xylose were filter sterilized separately from the fermentor and added to the fermentor with the inoculum (to avoid Maillard reactions). A 10% v/v inoculum was transferred to the fermentor vessel and was allowed to grow for 24 hours in batch mode before being switched to continuous operation. The effluent from the first stage was directed to the second stage (Figure 1). The feed for the continuous mode consisted of the same medium as the reactor, but was made up in a 15-L vessel with xylose and glucose being filter sterilized and added after the yeast extract and CSL solution was autoclaved.

The second stage was sterilized with enough water to cover the pH probe membrane. After sterilization and before the effluent line from the first stage was connected, a majority of the water was pumped out of the fermentor. The residence for the first stage was set at 24 hours; hence, once the second stage was attached to the system, it took 24 hours to fill it to the one-liter working volume.

The feed, base, and acid addition vessels were placed on balances and the weights were recorded daily in order to calculate the dilution rate for each fermentation and the overall dilution rate. The dilution rate was calculated by dividing the weight change over time of the feed (the base addition was negligible) by the working volume of the fermentor (density of feed assumed to be 1.0 g/mL). The residence time is the inverse of the dilution rate.

Figure 1: Schematic of two-stage continuous cofermentation set-up.



Sampling and Analysis

Samples were withdrawn at regular intervals and analyzed on the Yellow Springs Instrument (YSI) for ethanol and glucose. In addition, samples were analyzed by the Chemical Analysis and Testing (CAT) Team for glucose, xylose, apparent xylitol, acetic acid, lactic acid, and glycerol by HPLC and ethanol by GC. Optical density at 600 nm (OD) and dry cell weight were obtained on every sample to monitor cell growth. The dry cell weight was determined by centrifuging 4 mL of the fermentation broth in duplicate for 10 minutes at 5000 rev/min. The supernatant was decanted and the pellet was washed with 10 mL of deionized water twice. The pellets were then transferred to weighed pans and let to dry in a 60°C drying oven for 24 hours.

Results and Discussion

Stage One

In a previous one stage continuous cofermentation experiment with LNH33, 74 hour, 60 hour and 44 hour residence times, were examined. Since the glucose was completely consumed within the 44 hour residence time portion of that experiment [see Report 1.2, *Continuous Fermentation of Pure Sugars by L1400(pLNH33)*], the residence time of the first stage of this experiment was set at 24 hours to examine glucose and xylose utilization at an even shorter residence time (higher throughput).

In batch mode, glucose was consumed within eight hour. Within 24 hours, xylose dropped to 13.2 g/L from an original concentration of 35.4 g/L. This profile matches the previous profile observed with LNH33 (Table 1). After 24 hours of growth in batch mode, the fermentation was switched to continuous operation with a feed rate of 0.694 mL/min to yield a 24 hour residence time.

Table 1: Comparison of the sugar concentration profiles during the batch phase of the previous experiment (Batch 1) [Report 1.2] and this experiment (Batch 2).

	Glucose (g/L)		Xylose (g/L)	
Time (h)	Batch 1	Batch 2	Batch 1	Batch 2
0	25.51	25.50	38.44	35.40
3	21.18	19.89	38.62	35.78
6	4.43	3.68	37.77	35.47
24	0.0	0.0	15.8	13.62

After the fermentation was switched to continuous, the glucose concentration remained zero, but the xylose concentration increased over 265 hours of fermentation to 31.2 g/L. This represents a 3.98 g/L or a 11.3% conversion (Table 2) of xylose at a 24 hour residence time in the first stage. A decrease in xylose use resulted in a decrease in ethanol, glycerol, apparent xylitol, and dry cell weight production (Figures 2 and 3).

The ethanol and by-product yields and the glucose and xylose conversions for stage one were calculated based on the data from the initial and final time points of the experiment. It should be noted that the final time point represents the closest point available to a steady state at a 24 hour residence time (Figures 1 and 2). Even after 11 residence times, the fermentation had not fully reached a steady state. This may be due to an instability of the plasmid causing a decrease in the xylose utilization. The metabolic ethanol yield (based on consumed sugars) for stage 1 at a 24 hour residence time was 84.2% of theoretical, whereas the ethanol process yield (based on the available fermentable sugars) was only 40.0% of theoretical (Table 2) due to minimal xylose consumption.

The overall carbon balance closure was excellent at 97.58%. The major expenditure of carbon was to ethanol and carbon dioxide (CO_2) with a small amount going to cell mass, glycerol, and apparent xylitol (Table 3). The peak identified as apparent xylitol by HPLC has a slightly different retention time than xylitol and succinic acid, which elute very close to each other. One possibility is that the compound identified as apparent xylitol may be xylulose which is another product produced from the utilization of

xylose by LNH33. To determine the identity of this peak, xylulose, xylitol and apparent xylitol standards will be run together on the HPLC in conjunction with a sample that contains a peak identified as apparent xylitol.

Table 2: Fermentation Performance at a 24-hour residence time

Stage	1	2	Overall	One Stage Process
Residence Time	24	24	48	44
C6-Conversion:	100.0%	-	100.0	99.3%
C5-Conversion:	11.3%	16.9%	26.3%	27.1%
Ethanol Process Yield (% theoretical):	40.0%	14.4%	47.6%	46.8%
Ethanol Metabolic Yield (% theoretical):	84.2%	85.2%	84.4%	82.0%

Table 3: Product Distribution

	g product/100 g C6+C5 consumed		
	Stage 1	Stage 2	Overall
Ethanol	43.06	43.56	43.14
Cell Mass	8.08	4.55	7.53
Carbon Dioxide	41.14	41.61	41.21
Glycerol	2.82	6.44	3.39
Acetic Acid	0.00	1.52	.21
Lactic Acid	0.00	0.00	0.00
Apparent xylitol	2.47	18.18	4.94
Total	97.58	115.86	100.42

Stage Two and Overall System

Stage two was completely filled and operational after 52 hours from the time stage one was inoculated. The only fermentable sugar available in stage two was xylose, as all of the glucose was consumed in the first stage. The xylose concentration in stage two decreased to 6.8 g/L soon after the vessel had been filled and increased to 25.96 g/L by the end of the fermentation. This represents a xylose conversion of 16.9% at a 24 hour residence time in stage two, for an overall process conversion through both stages of 26.3% (Table 2) (48 hour residence time). In the previous one stage continuous cofermentation with LNH33 the xylose conversion was 27.1% (Table 2) at a 44 hour residence time. It is interesting that the xylose conversion rates of the one-stage and two-stage systems are comparable at similar residence times.

The ethanol process yield in the second stage was a low 14.4% of theoretical, whereas the ethanol metabolic yield was 85.2% of theoretical. For the overall process, the ethanol process yield was 47.6% and the ethanol metabolic yield was 84.4%. Again, these results compare well with the results obtained from

the one stage continuous cofermentation that had an ethanol process yield of 46.8% and an ethanol metabolic yield of 82.0% (Table 2). Besides CO_2, the major byproducts in stage two were the same as in stage one: apparent xylitol and glycerol (Table 3).

Conclusions

All the glucose present in the first stage was consumed rapidly within eight hours in batch mode, whereas. 61.5% of the xylose was consumed in 24 hours of batch operation. However, when the fermentation was switched to continuous operation with a 24 hour residence time, the xylose concentration increased to 31.24 g/L representing only an 11.3% conversion of xylose; at the same time, the glucose concentration remained at zero. The second stage witnessed an additional 14.4% conversion of xylose at a 24-hour residence time for an overall xylose conversion of 26.3% at a 48-hour residence time. The inability of the system to reach a steady state with respect to xylose within 11 residence times may be due to the instability of the plasmid that carried the xylose-catabolism genes.

The ethanol metabolic yields were good in both stages at 84.2% theoretical in stage one and 85.2% theoretical in stage two for an overall yield of 84.4% theoretical. The major byproducts besides CO_2 were cell mass, glycerol, and apparent xylitol.

The overall results obtained from the two stage continuous cofermentation are not superior to those obtained with a single stage continuous cofermentation performed under identical conditions (Report 1.2). This would suggest that a two-stage system were the glucose is consumed in the first stage and the second stage only sees xylose does not result in a significant improvement in the overall process yields, when LNH33 is the ethanologenic organism.

Figure 2: Sugar Utilization and Ethanol Production for Two-Stage Continuous Cofermentation with L1400(pLNH33)

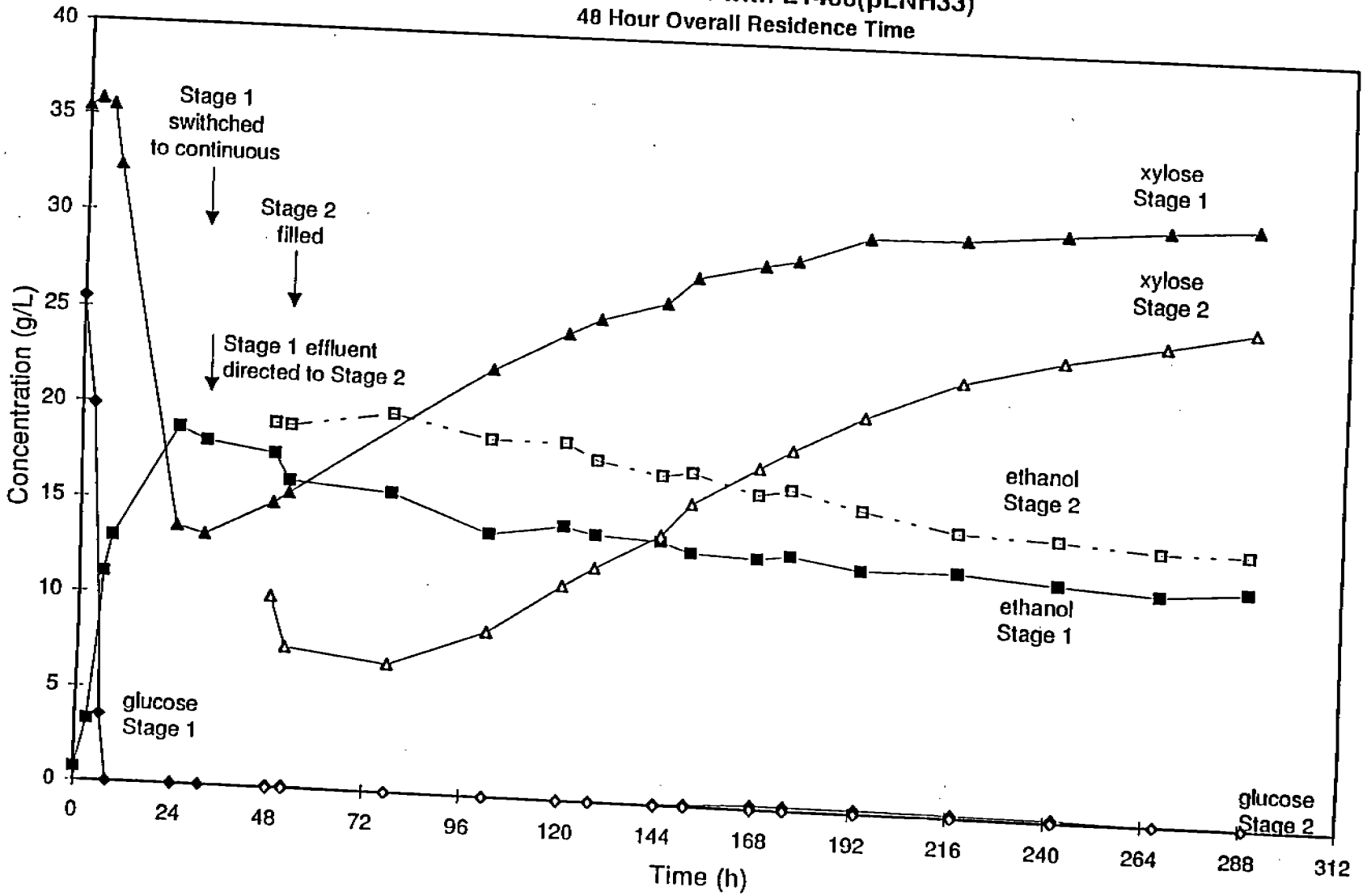
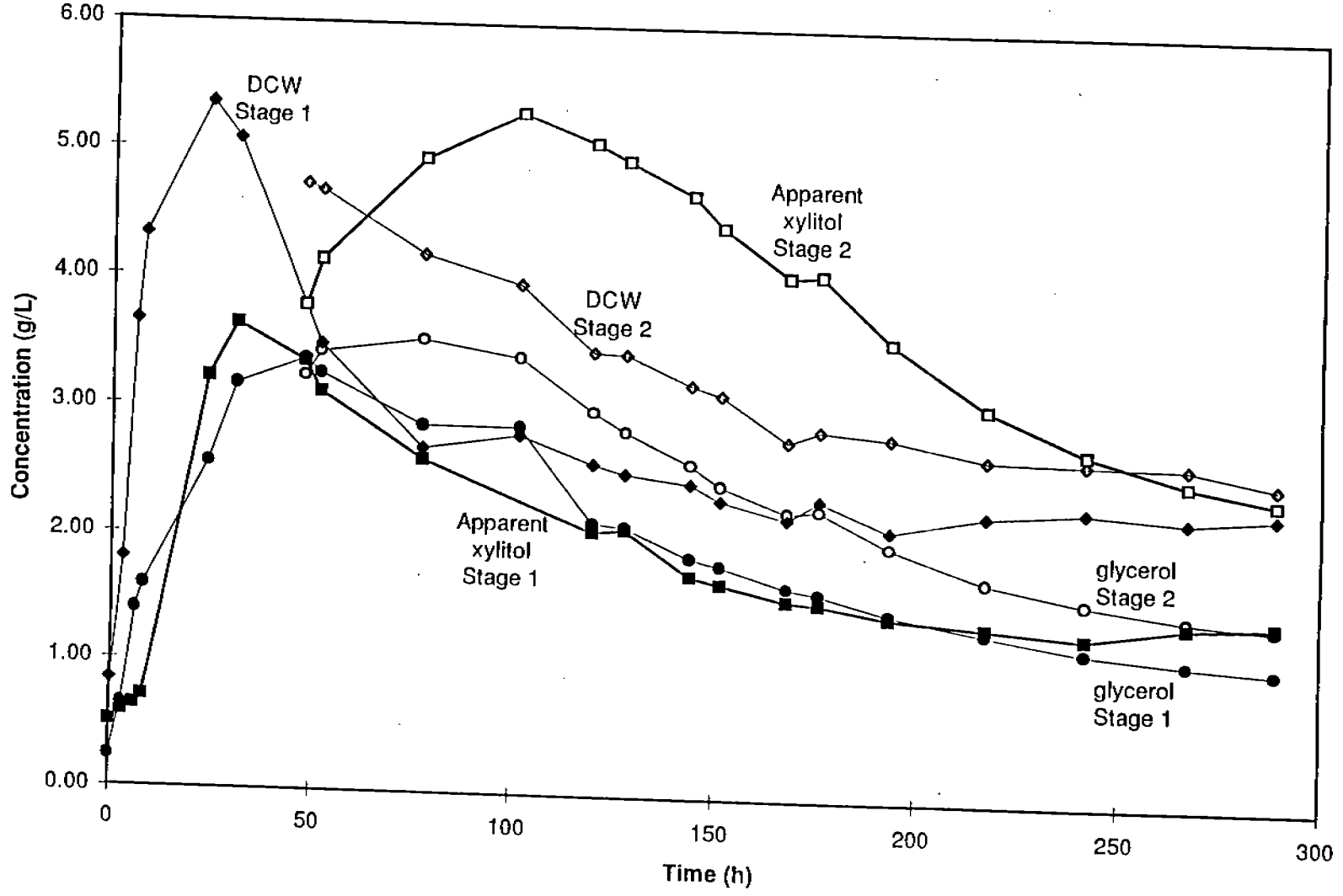


Figure 3: By-Product Profile for Two-Stage Continuous Cofermentation with L1400(pLNH33)
48 Hour Overall Residence Time



Raw Data

		Stage 1											
				YSI	LC	YSI	GC						
Sample	Time elapsed (h)	OD (600 nm)	DCW (g/L)	Glucose (g/L)	Glucose (g/L)	Ethanol (g/L)	Ethanol (g/L)	Xylose (g/L)	Xylitol (g/L)	Glycerol (g/L)	Lactic acid (g/L)	Succinic acid (g/L)	acetic acid (g/L)
1	0	1.40	0.84	26.70	25.50	0.71	0.90	35.40	ND	0.24	2.89	0.51	0.18
2	3	2.45	1.80	19.50	19.89	3.31	3.50	35.78	ND	0.65	2.91	0.59	0.31
3	6	5.36	3.66	3.68	3.56	11.06	10.50	35.47	ND	1.40	2.88	0.64	0.23
4	8	6.81	4.32	0.07	0.00	13.00	13.00	32.36	ND	1.59	2.67	0.71	0.00
5	24	8.74	5.36	0.03	0.00	18.78	20.20	13.62	ND	2.56	2.37	3.22	0.01
6	31	7.58	5.08	0.05	0.00	18.12		13.24	ND	3.17	2.88	3.64	0.06
7	48	5.76	3.79	0.12	0.08	17.58		15.01	ND	3.37	3.00	3.35	0.00
8	52	5.31	3.49	0.19	0.10	16.20		15.58	ND	3.26	2.89	3.12	0.00
9	77.5	3.86	2.70	0.25	0.00	15.78			ND	2.87	2.84	2.61	0.00
10	101.75	3.92	2.81	0.20	0.00	13.86		22.46	ND	2.87	2.86		0.00
11	120	3.78	2.60	0.19	0.00	14.42		24.55	ND	2.14	2.78	2.07	0.00
12	128	3.72	2.53	0.05	0.02	14.06		25.36	ND	2.11	2.88	2.09	0.00
13	144	3.77	2.46	0.24	0.04	13.86	13.90	26.32	ND	1.88	2.78	1.74	0.00
14	151.5	3.62	2.34	0.19	0.10	13.32	13.70	27.67	ND	1.82	2.81	1.68	0.00
15	168	3.60	2.20	0.31	0.20	13.16	13.60	28.42	ND	1.66	2.80	1.56	0.00
16	176	3.68	2.35	0.30	0.21	13.36	13.10	28.73	ND	1.62	2.87	1.54	0.00
17	193.5	3.44	2.12	0.32	0.21	12.76	12.60	30.06	ND	1.47	2.84	1.44	0.00
18	217.5	3.67	2.25	0.23	0.15	12.84	12.70	30.17	ND	1.33	2.87	1.38	0.00
19	242	3.60	2.30	0.27	0.16	12.39	12.80	30.61	ND	1.20	2.81	1.31	0.00
20	267	3.60	2.24	0.27	0.00	12.06	12.40	31.01	ND	1.12	2.81	1.42	0.00
21	289	3.65	2.29	0.15	0.00	12.34	12.20	31.24	ND	1.07	2.82	1.44	0.07
ND - Not detected													

Raw Data

		Stage 2											
				YSI	LC	YSI	GC						
Sample	Time elapsed (h)	OD (600 nm)	DCW (g/L)	Glucose (g/L)	Glucose (g/L)	Ethanol (g/L)	Ethanol (g/L)	Xylose (g/L)	Xylitol (g/L)	Glycerol (g/L)	Lactic acid (g/L)	Succinic acid (g/L)	acetic acid (g/L)
1	0												
2	3												
3	6												
4	8												
5	24												
6	31												
7	48	7.09	4.73	0.04	0.00	19.18		10.09	ND	3.24	2.75	3.79	0.00
8	52	7.14	4.68	0.04	0.00	19.12		7.49	ND	3.43	2.80	4.14	0.00
9	77.5	4.78	4.19	0.03	0.00	19.90		6.79	ND	3.54	2.80	4.94	0.08
10	101.75	5.21	3.98	0.04	0.00	18.78		8.70	ND	3.41	2.90	5.31	0.11
11	120	4.84	3.46	0.04	0.00	18.78		11.28	ND	3.00	2.82	5.08	0.10
12	128	4.90	3.45	0.00	0.00	17.94		12.30	ND	2.85	2.81	4.95	0.10
13	144	4.54	3.23	0.00	0.00	17.31	18.60	14.18	ND	2.61	2.79	4.69	0.10
14	151.5	4.54	3.15	0.04	0.00	17.50	18.00	15.89	ND	2.45	2.75	4.44	0.09
15	168	4.28	2.80	0.04	0.00	16.50	16.70	17.89	ND	2.25	2.78	4.07	0.10
16	176	4.37	2.89	0.04	0.00	16.82	16.60	18.88	ND	2.27	2.92	4.09	0.10
17	193.5	4.30	2.84	0.04	0.00	15.88	16.60	20.75	ND	1.99	2.81	3.58	0.09
18	217.5	4.28	2.69	0.08	0.00	14.92	15.80	22.79	ND	1.73	2.76	3.08	0.09
19	242	4.27	2.68	0.01	0.00	14.71	15.00	24.06	ND	1.58	2.76	2.75	0.12
20	267	4.44	2.66	0.04	0.00	14.30	14.70	25.03	ND	1.47	2.79	2.53	0.13
21	289	4.21	2.53	0.05	0.00	14.28	14.50	25.96	ND	1.41	2.81	2.40	0.15
ND - Not detected													

Feed Concentration Analysis							
Sample	Time Taken (h)	Glucose (g/L)	Xylose (g/L)	Glycerol (g/L)	Lactic acid (g/L)	Apparent Xylitol (g/L)	Acetic Acid (g/L)
1a	0	24.5	34.67	0.36	3.70	1.25	0.07
1b	120	24.86	35.09	0.15	2.89	0.17	0.08
1c	218	24.01	35.54	0.15	2.86	0.17	0.08
1d	289	24.03	35.56	0.41	3.85	1.38	0.08
Average		24.35	35.22	0.27	3.33	0.74	0.08

CARBON BALANCE: L1400(pLNH33) Continuous Fermentation with Pure Sugars

Sample: Stage 1 - 24 hour residence time
Pretreatment:
Run:

SOLIDS BALANCE	In	Out
Lignin (%):	0	0.00
Insoluble Solids (%):	0.00	0.00

Cellulose Conversion:	#DIV/0!
Overall C6-Sugar Conversion:	100.0%
Overall C5-Sugar Conversion:	11.3%
Ethanol Process Yield (% theor):	40.0%
Ethanol Metabolic Yield (% theor):	84.2%

Carbon Balance: SSF

	Carbon In							Carbon Out									
	In Solids			In Liquor			Total	In Solids			In Liquor			Total	Conversion	Yield	Yield
Component	(% dry wt)	(C-mole/Kg Sln	(% Total In)	(g/L)	(C-mole/Kg Sln	(% Total In)	(C-mole/Kg Sln)	(% dry wt)	(C-mole/Kg Sln	% Total Out)	(g/L)	(C-mole/Kg Sln	% Total Out)	(C-mole/Kg Sln	(In-Out)/In (%)	g product/ 100 g C6 cons	g product/ 100 g C6+C5 cons
Cellobiose				0.00	0.000		0.000				0.00	0.000		0.000			
Glucose	0	0.000	0.0	24.35	0.811	100.0	0.811	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	100.00		
Galactose	0	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Mannose	0	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Xylose	0	0.000	0.0	35.22	1.173	100.0	1.173	0.00	0.000	0.0	31.24	1.040	100.0	1.040	11.30		
Arabinose	0	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Lignin	0	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Ethanol	0			0.00	0.000		0.000				12.20	0.530		0.530		50.10	43.06
Cell Mass				0.00	0.000		0.000				2.29	0.091		0.091		9.40	8.08
Carbon Dioxide				0.00			0.000					0.265		0.265		47.86	41.14
Glycerol				0.27	0.009		0.009				1.07	0.035		0.035		3.29	2.82
Acetic Acid				0.08	0.003		0.003				0.07	0.002		0.002		-0.04	0.00
Lactic Acid				3.33	0.111		0.111				2.82	0.094		0.094		-2.09	0.00
Succinic Acid				0.74	0.025		0.025				1.44	0.049		0.049		2.87	2.47
Xylitol				0.00	0.000		0.000				0.00	0.000		0.000		0.00	0.00
Other																	
Total	0	0.000	0.0		2.131	100.0	2.131	0.00	0.000	0.0		2.106	100.0	2.106		111.39	97.58

C - Recovery: 98.82%

Component	% Carbon In	% Carbon Out
Glucose	38.0%	0.0%
Galactose+Mannose	0.0%	0.0%
Total C5 Sugars	55.0%	49.4%
Lignin	0.0%	0.0%
Ethanol	0.0%	25.1%
Byproducts	6.9%	25.5%

CARBON BALANCE: L1400(pLNH33) Continuous Fermentation with Pure Sugars

Sample: Overall - 48 hr residence time
Pretreatment:
Run:

SOLIDS BALANCE	In	Out
Lignin (%):	0	0.00
Insoluble Solids (%):	0.00	0.00

Cellulose Conversion:	#DIV/0!
Overall C6-Sugar Conversion:	100.0%
Overall C5-Sugar Conversion:	26.3%
Ethanol Process Yield (% theor):	47.6%
Ethanol Metabolic Yield (% theor):	84.4%

Carbon Balance: SSF

	Carbon In							Carbon Out							Conversion	Yield	Yield
	In Solids			In Liquor			Total	In Solids			In Liquor			Total	(In-Out)/In	g product/	g product/
Component	(% dry wt)	(C-mole/Kg Sln	(% Total In)	(g/l)	(C-mole/Kg Sln	(% Total In)	(C-mole/Kg Sln)	(% dry wt)	(C-mole/Kg Sln	% Total Out)	(g/l)	(C-mole/Kg Sln	% Total Out)	(C-mole/Kg Sln	(%)	100 g C6 cons	100 g C6+C5 cons
Cellobiose																	
Glucose	0	0.000	0.0	0.00	0.000		0.000				0.00	0.000		0.000			
Galactose	0	0.000	#DIV/0!	24.35	0.811	100.0	0.811	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	100.00		
Mannose	0	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Xylose	0	0.000	0.0	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Arabinose	0	0.000	#DIV/0!	35.22	1.173	100.0	1.173	0.00	0.000	0.0	25.96	0.865	100.0	0.865	26.29		
				0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Lignin	0	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Ethanol	0																
Cell Mass				0.00	0.000		0.000				14.50	0.629		0.629		59.55	43.14
Carbon Dioxide				0.00	0.000		0.000				2.53	0.101		0.101		10.39	7.53
Glycerol				0.00								0.315		0.315		56.89	41.21
Acetic Acid				0.27	0.009		0.009				1.41	0.046		0.046		4.68	3.39
Lactic Acid				0.08	0.003		0.003				0.15	0.005		0.005		0.29	0.21
Succinic Acid				3.33	0.111		0.111				2.81	0.094		0.094		-2.14	0.00
Xylitol				0.74	0.025		0.025				2.40	0.081		0.081		6.82	4.94
Other				0.00	0.000		0.000				0.00	0.000		0.000		0.00	0.00
Total	0	0.000	0.0		2.131	100.0	2.131	0.00	0.000	0.0		2.136	100.0	2.136		136.48	100.42

C- RECOVERY: 101.20%

Component	% Carbon In	% Carbon Out
Glucose	38.0%	0.0%
Galactose+Mannose	0.0%	0.0%
Total C5 Sugars	55.0%	40.5%
Lignin	0.0%	0.0%
Ethanol	0.0%	29.5%
Byproducts	6.9%	30.0%

CARBON BALANCE: L1400(pLNH33) Continuous Fermentation with Pure Sugars

Sample: Stage 2 - 24 hour residence time
Pretreatment:
Run:

SOLIDS BALANCE	In	Out
Lignin (%):	0	0.00
Insoluble Solids (%):	0.00	0.00

Cellulose Conversion:	#DIV/0!
Overall C6-Sugar Conversion:	#DIV/0!
Overall C5-Sugar Conversion:	16.9%
Ethanol Process Yield (% theor):	14.4%
Ethanol Metabolic Yield (% theor):	85.2%

Carbon Balance: SSF

Component	Carbon In: In Solids (% dry wt)	(C-mole/Kg Sln	(% Total In)	In Liquor (g/L)	(C-mole/Kg Sln	(% Total In)	Total (C-mole/Kg Sln)	Carbon Out: In Solids (% dry wt)	(C-mole/Kg Sln	% Total Out)	In Liquor (g/L)	(C-mole/Kg Sln	% Total Out)	Total (C-mole/Kg Sln)	Conversion (In-Out)/In (%)	Yield g product/ 100 g C6 cons	Yield g product/ 100 g C6+C5 cons
Cellobiose	0	0.000	#DIV/0!	0.00	0.000		0.000				0.00	0.000		0.000			
Glucose	0	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Galactose	0	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Mannose	0	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Xylose	0	0.000	0.0	31.24	1.040	100.0	1.040	0.00	0.000	0.0	25.96	0.865	100.0	0.865	16.90		
Arabinose	0	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Lignin	0	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000										
Ethanol	0							0.00	0.000	#DIV/0!	0.00	0.000	#DIV/0!	0.000	#DIV/0!		
Cell Mass				12.20	0.530		0.530				14.50	0.629		0.629		#DIV/0!	43.56
Carbon Dioxide				2.29	0.091		0.091				2.53	0.101		0.101		#DIV/0!	4.55
Glycerol				0.00								0.050		0.050		#DIV/0!	41.61
Acetic Acid				1.07	0.035		0.035				1.41	0.046		0.046		#DIV/0!	6.44
Lactic Acid				0.07	0.002		0.002				0.15	0.005		0.005		#DIV/0!	1.52
Succinic Acid				2.82	0.094		0.094				2.81	0.094		0.094		#DIV/0!	0.00
Xylitol				1.44	0.049		0.049				2.40	0.081		0.081		#DIV/0!	18.18
Other				0.00	0.000		0.000				0.00	0.000		0.000		#DIV/0!	0.00
Total	*0*	0.000	*0.0*		1.841	*100.0*	*1.841*	*0.00*	0.000	*0.0*		1.871	*100.0*	*1.871*		#DIV/0!	115.86

C- Recovery: 101.60%

Component	% Carbon In	% Carbon Out
Glucose	0.0%	0.0%
Galactose+Mannose	0.0%	0.0%
Total C5 Sugars	56.5%	46.2%
Lignin	0.0%	0.0%
Ethanol	28.8%	33.6%
Byproducts	14.7%	20.1%